

What is claimed is:

- 1 1. A recombinant DNA comprising said DNA selected from the group consisting of:
  - 2 a) a recombinant DNA that encodes a protein having an amino acid
  - 3 sequence as shown in SEQ. ID. NO. 3;
  - 4 b) a recombinant DNA that encodes a protein having an amino acid
  - 5 sequence as shown in SEQ. ID. NO. 5;
  - 6 c) a recombinant DNA that encodes a protein having an amino acid
  - 7 sequence as shown in SEQ. ID. NO. 7;
  - 8 d) a recombinant DNA that encodes a protein having an amino acid
  - 9 sequence as shown in SEQ. ID. NO. 9;
  - 10 e) a recombinant DNA that encodes a protein having an amino acid
  - 11 sequence as shown in SEQ. ID. NO. 11; and
  - 12 f) any portion of said DNA above that encodes a protein that elicits an
  - 13 immune response against *E. canis*.
- 1 2. The recombinant DNA of claim 1 wherein said DNA encodes at least one
- 2 immunogenic epitope.
- 1 3. A recombinant protein comprising said protein selected from the group consisting of:
  - 2 a) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;
  - 3 b) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;
  - 4 c) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;
  - 5 d) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;
  - 6 e) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

f) any portion of any of the above proteins that elicits an immune response against *E. canis*.

4. The recombinant protein of claim 3 wherein said protein includes at least one immunogenic epitope.

5. A vaccine wherein said vaccine protects dogs against *E. canis* infection.

6. A vaccine comprising:

a) a vector capable of expressing a recombinant DNA inserted into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host; and

b) the recombinant DNA inserted into said vector wherein said DNA is selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein fragment that is greater than 25 amino acids.

7. The vaccine of claim 6, wherein said DNA further comprises DNA that encodes CpG motifs.

1 8. The vaccine of claim 6 wherein said DNA further comprises a promoter selected from  
2 the group consisting of:

3 a) a cytomegalovirus (CMV) immediate early promoter;

4 b) a human tissue plasminogen activator gene (t-PA); and

5 c) promoter/enhancer region of a human elongation factor alpha (EF-1  $\alpha$ ).

1 9. The vaccine of claim 6, wherein said vector is selected from the group consisting of:

2 a) pcDNA3;

3 b) pC1;

4 c) VR1012; and

5 d) VR1020.

1 10. The vaccine of claim 6 wherein said vaccine is administered into said host by a  
2 method selected from the group consisting of:

3 a) intramuscular injection;

4 b) intravenous injection; and

5 c) gene gun injection.

1 11. The vaccine of claim 10, wherein said host is a dog.

1 12. The vaccine of claim 5 comprising:

2 a) a recombinant protein that is selected from the group consisting of:

3 i) a protein having an amino acid sequence as shown in SEQ. ID. NO.

4 3;

5 ii) a protein having an amino acid sequence as shown in SEQ. ID.

6 NO. 5;

iii) a protein having an amino acid sequence as shown in SEQ. ID.

NO. 7;

iv) a protein having an amino acid sequence as shown in SEQ. ID.

NO. 9;

v) a protein having an amino acid sequence as shown in SEQ. ID.

NO. 11; and

vi) any portion of any of the above proteins that elicits an immune response against *E. canis*.

13. The vaccine of claim 12, wherein said vaccine further comprises adjuvants selected from the group consisting of:

a) aluminum hydroxide;

b) QuilA; and

c) Montamide.

14. The vaccine of claim 12 further comprising a cytokine operatively associated with said recombinant protein.

15. The vaccine of claim 14 wherein said cytokine is selected from the group consisting of:

a) interleukin-1 $\beta$  (IL-1 $\beta$ );

b) granulocyte-macrophage colony stimulating factor (GM-CSF);

c) gamma interferon ( $\gamma$ -IFN);

d) amino acids VQGEESNDK from the IL-1 $\beta$  protein; and

e) any portion of any of the cytokines above that elicits an improved immunogenic response against *E. canis*.

1 16. The vaccine of claim 12 wherein said vaccine is administered into a host by a method  
2 selected from the group consisting of:

3 a) intramuscular injection; and

4 b) subcutaneous injection.

1 17. The vaccine of claim 16 wherein said host is a dog.

1 18. The vaccine of claim 5 comprising a recombinant protein that includes a T cell epitope  
2 wherein said T cell epitope comprises an amino acid peptide fragment of a protein  
3 selected from the group consisting of:

4 a) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

5 b) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

6 c) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

7 d) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

8 e) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11;

9 and

10 f) any portion of any of the above proteins that elicits an immune response  
11 against *E. canis*.

1 19. The vaccine of claim 18 wherein said amino acid peptide fragment comprises nine to  
2 twenty amino acids.

1 20. The vaccine of claim 18 further comprising a recombinant DNA encoding a protein  
2 which is capable of being internalized into eukaryotic cells, including cells of the  
3 immune system.

1 21. The vaccine of claim 20 wherein said protein capable of being internalized into  
2 eukaryotic cells comprises a toxin selected from the group consisting of:

3 a) a recombinant adenylate cyclase of *Bordetella bronchiseptica*; and

b) a recombinant exotoxin A (PE) of *Pseudomonas aeruginosa*.

22. The vaccine of claim 18 wherein said vaccine is administered into a host by a method selected from the group consisting of:

a) intramuscular injection; and

b) subcutaneous injection.

23. The vaccine of claim 22 wherein said host is a dog.

24. A method of identifying a T cell epitope against *E. canis* comprising:

a) synthesizing overlapping peptide fragments over an entire length of a protein wherein said protein is selected from the group consisting of:

i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of any of the proteins above that elicits an immune response against *E. canis*;

b) testing said peptide fragment to determine if said peptide fragment elicits an immune response in a host animal; and

c) identifying said peptide fragment as said T cell epitope of *E. canis* if said fragment elicits an immune response.

25. The method of claim 24 wherein said peptide fragment comprises nine to twenty amino acids.

26. A method of creating a vaccine against *Ehrlichia canis* comprising:

a) selecting a vector capable of expressing a recombinant DNA inserted into said vector; and

b) inserting a recombinant DNA into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host wherein said DNA is selected from the group consisting of:

i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and

vi) any portion of said DNA above that encodes a protein fragment that is greater than 25 amino acids.

27. The method of claim 26, wherein said DNA further comprises DNA that encodes CpG motifs.

1 28. The method of claim 26 wherein said DNA further comprises a promoter selected  
2 from the group consisting of:

3 a) a cytomegalovirus (CMV) immediate early promoter;

4 b) a human tissue plasminogen activator gene (t-PA); and

5 c) a promoter/enhancer region of a human elongation factor alpha (EF-1  $\alpha$ ).

1 29. The method of claim 26, wherein said vector is selected from the group consisting of:

2 a) pcDNA3;

3 b) pC1;

4 c) VR1012; and

5 d) VR1020.

1 30. The method of claim 26 wherein said vaccine is injected into said host in a manner  
2 selected from the group consisting of:

3 a) intramuscular injection;

4 b) intravenous injection; and

5 c) gene gun injection.

1 31. The method of claim 30, wherein said host is a dog.

1 32. A method of creating a vaccine against *E. canis* comprising:

2 a) selecting a vector capable of expressing a recombinant protein inserted  
3 into said vector;

4 b) insertion of a recombinant DNA into said vector such that said  
5 recombinant protein is expressed when said vector is transformed  
6 into a bacterial strain wherein said DNA is selected from the group  
7 consisting of:



- 8 i) a recombinant DNA that encodes a protein having an amino acid  
9 sequence as shown in SEQ. ID. NO. 3;
- 10 ii) a recombinant DNA that encodes a protein having an amino acid  
11 sequence as shown in SEQ. ID. NO. 5;
- 12 iii) a recombinant DNA that encodes a protein having an amino acid  
13 sequence as shown in SEQ. ID. NO. 7;
- 14 iv) a recombinant DNA that encodes a protein having an amino acid  
15 sequence as shown in SEQ. ID. NO. 9;
- 16 v) a recombinant DNA that encodes a protein having an amino acid  
17 sequence as shown in SEQ. ID. NO. 11; and
- 18 vi) any portion of said DNA above that encodes a protein that elicits  
19 an immune response against *E. canis*; and
- 20 c) harvesting said recombinant protein from said bacterial strain.

1 33. The method of claim 32, wherein said vaccine further comprises adjuvants selected  
2 from the group consisting of:

- 3 a) aluminum hydroxide;
- 4 b) QuilA; and
- 5 c) Montamide.

1 34. The method of claim 32, wherein said vaccine further comprises a promoter selected  
2 from the group consisting of:

- 3 a) tac;
- 4 b) T5; and
- 5 c) T7.

- 1 35. The method of claim 32, wherein said bacterial strain is *E. coli*.
- 1 36. The method of claim 32, wherein said vector is selected from the group consisting of:
- 2 a) pREST;
- 3 b) pET; and
- 4 c) pKK233-3.
- 1 37. The method of claim 32 wherein said vaccine further comprises a cytokine operatively  
2 associated with said vaccine.
- 1 38. The method of claim 37 wherein said cytokine is selected from the group consisting  
2 of:
- 3 a) interleukin-1 $\beta$  (IL-1 $\beta$ );
- 4 b) granulocyte-macrophage colony stimulating factor (GM-CSF);
- 5 c) gamma interferon ( $\gamma$ -IFN);
- 6 d) amino acids VQGEESNDK from the IL-1 $\beta$  protein; and
- 7 e) any portion of any of the cytokines above that elicits an improved  
8 immunogenic response against *E. canis*.
- 1 39. The method of claim 32 wherein said vaccine is injected into said host in a manner  
2 selected from the group consisting of:
- 3 a) intramuscular injection; and
- 4 b) subcutaneous injection.
- 1 40. The method of claim 39 wherein said host is a dog.
- 1 41. A method of creating a T cell epitope vaccine comprising:

2 a) selecting a recombinant protein that includes a T cell epitope wherein  
3 said T cell epitope comprises an amino acid peptide fragment of a  
4 protein selected from the group consisting of:

5 i) a protein having an amino acid sequence as shown in SEQ. ID. NO.  
6 3;

7 ii) a protein having an amino acid sequence as shown in SEQ. ID.  
8 NO. 5;

9 iii) a protein having an amino acid sequence as shown in SEQ. ID.  
10 NO. 7;

11 iv) a protein having an amino acid sequence as shown in SEQ. ID.  
12 NO. 9;

13 v) a protein having an amino acid sequence as shown in SEQ. ID.  
14 NO. 11; and

15 vi) any portion of any of the above proteins that elicits an immune  
16 response against *E. canis*;

17 b) identifying said T cell epitope from said protein;

18 c) incorporating said T cell epitope into a construct capable of expressing  
19 said epitope as a protein; and

20 d) harvesting said protein.

1 42. The method of claim 41 wherein said amino acid peptide fragment comprises nine to  
2 twenty amino acids.

1 43. The method of claim 41 wherein said construct capable of expressing said epitope  
2 further comprises a recombinant DNA encoding a protein which is capable of  
3 being internalized into eukaryotic cells, including cells of the immune system.

1 44. The method of claim 43 wherein said protein capable of being internalized into  
2 eukaryotic cells comprises a toxin selected from the group consisting of:

3 a) a recombinant adenylate cyclase of *Bordetella bronchiseptica*; and

4 b) a recombinant exotoxin A (PE) of *Pseudomonas aeruginosa*.

1 45. The method of claim 41 wherein said vaccine is injected into said host in a manner  
2 selected from the group consisting of:

3 a) intramuscular injection; and

4 b) subcutaneous injection.

1 46. The method of claim 45 wherein said host is a dog.

1 47. A recombinant DNA comprising said DNA selected from the group consisting of

2 a) a recombinant DNA that encodes a protein having an amino acid  
3 sequence as shown in SEQ. ID. NO. 3;

4 b) a recombinant DNA that encodes a protein having an amino acid  
5 sequence as shown in SEQ. ID. NO. 5;

6 c) a recombinant DNA that encodes a protein having an amino acid  
7 sequence as shown in SEQ. ID. NO. 7;

8 d) a recombinant DNA that encodes a protein having an amino acid  
9 sequence as shown in SEQ. ID. NO. 9; and

10 e) a recombinant DNA that encodes a protein having an amino acid  
11 sequence as shown in SEQ. ID. NO. 11.

1 48. A vector capable of expressing a recombinant DNA comprising:

2 a) a recombinant DNA inserted into said vector such that a recombinant  
3 protein is expressed when said vector is provided in an appropriate  
4 host wherein said DNA is selected from the group consisting of:

- i) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;
- ii) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;
- iii) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;
- iv) a recombinant DNA sequence that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9;
- v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11; and
- vi) any portion of said DNA above that encodes a protein that elicits an immune response against *E. canis*.

49. The recombinant DNA of claim 47 wherein said DNA encodes at least one immunogenic epitope.

50. A vector capable of expressing a recombinant DNA comprising:

- a) a recombinant DNA inserted into said vector such that a recombinant protein is expressed when said vector is provided in an appropriate host wherein said DNA is selected from the group consisting of:
  - i) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;
  - ii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;
  - iii) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

iv) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9; and

v) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11.

51. Serological diagnosis techniques using:

a) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

b) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

c) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 7;

d) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 9; and

e) a recombinant DNA that encodes a protein having an amino acid sequence as shown in SEQ. ID. NO. 11.

52. The method of kinetic enzyme-linked immunosorbent assay comprising the steps of:

a) selecting an antigen to be added to microtiter plates that includes an immunogenic epitope comprising a recombinant protein selected from the group consisting of:

i) a protein having an amino acid sequence as shown in SEQ. ID. NO. 3;

ii) a protein having an amino acid sequence as shown in SEQ. ID. NO. 5;

iii) a protein having an amino acid sequence as shown in SEQ. ID.  
NO. 7;

iv) a protein having an amino acid sequence as shown in SEQ. ID.  
NO. 9;

v) a protein having an amino acid sequence as shown in SEQ. ID.  
NO. 11;

vi) any portion of said DNA above that encodes a protein that elicits  
an immune response against *E. canis*

b) adding an antiserum of the species allowing it to complementarily bind  
to the antigen;

c) adding the antibody to the microtiter plate, allowing the antibody to bind  
to the antigen;

d) washing the microtiter plate to remove any unbound antibodies;

e) adding an enzyme the microtiter plates allowing the enzyme to bind to  
the antibody;

f) washing the microtiter plate to remove any unbound enzyme; and

g) adding the enzyme's substrate, allowing it to bind to the enzyme, which  
produces a color change when bound.

53. The method of claim 52, where said species is a canine.

54. The method of claim 52, wherein antiserum added to the microtiter plate is goat anti-  
canine.

55. The method of claim 52, wherein the antibody added to the microtiter plate is second  
antibodies of a goat anti-canine antibody of heavy and light chain specificity.

56. The method of claim 52, wherein the enzyme added to the microtiter plate is  
horseradish peroxidase.

1 57. The method of claim 52, wherein the enzyme's substrate is chromogen  
2 tetramethylbenzidine with H<sub>2</sub>O<sub>2</sub>.

1 58. The method of western blot analysis comprising the steps of:

2 a) obtaining the species serum with antigens, where said antigen includes  
3 an immunogenic epitope comprising a recombinant protein selected  
4 from the group consisting of;

5 i) a protein having an amino acid sequence as shown in SEQ. ID. NO.  
6 3;

7 ii) a protein having an amino acid sequence as shown in SEQ. ID.  
8 NO. 5;

9 iii) a protein having an amino acid sequence as shown in SEQ. ID.  
10 NO. 7;

11 iv) a protein having an amino acid sequence as shown in SEQ. ID.  
12 NO. 9;

13 v) a protein having an amino acid sequence as shown in SEQ. ID.  
14 NO. 11;

15 vi) any portion of said DNA above that encodes a protein that elicits  
16 an immune response against *E. canis*

17 b) running the serum through sodium dodecyl sulfate-polyacrylamide gel  
18 electrophoresis, allowing proteins to be fractionated into a series of  
19 bands arranged in order of molecular weight;

20 c) transferring the proteins to a filter by blotting;

21 d) adding antibodies tagged with a dye are washed over the filter, allowing  
22 the antibodies to bind to the fractionated proteins; and

23 e) adding substrates to develop the bands on the filter.



- 1 59. The method of claim 58, wherein said species is a canine.
- 1 60. The method of claim 58, wherein the antibodies are goat anti-dog igG conjugated to  
2 horseradish peroxidase.
- 1 61. The method of claim 58, wherein the substrates added to develop the bands on the  
2 filter are:
- 3 a) 4 chloro-1-naphthol in methyl alcohol;
- 4 b) tris-buffer solution with a pH of 7.5; and
- 5 c) 30% H<sub>2</sub>O<sub>2</sub>.
- 1 62. The method of polymerase chain reaction comprising the steps of:
- 2 a) selecting a target strand of DNA that will serve as a template for DNA  
3 synthesis comprising recombinant DNA selected from the group  
4 consisting of:
- 5 i) a recombinant DNA that encodes a protein having an amino acid  
6 sequence as shown in SEQ. ID. NO. 3;
- 7 ii) a recombinant DNA that encodes a protein having an amino acid  
8 sequence as shown in SEQ. ID. NO. 5;
- 9 iii) a recombinant DNA that encodes a protein having an amino acid  
10 sequence as shown in SEQ. ID. NO. 7;
- 11 iv) a recombinant DNA that encodes a protein having an amino acid  
12 sequence as shown in SEQ. ID. NO. 9;
- 13 v) a recombinant DNA that encodes a protein having an amino acid  
14 sequence as shown in SEQ. ID. NO. 11; and
- 15 vi) any portion of said DNA above that encodes a protein that elicits  
16 an immune response against *E. canis*;
- 10004494-10201

- 17 b)adding a mixture containing enzymes, nucleotides, DNA polymerase, and  
18 primers;
- 19 c)subjecting above mixture to a number of cycles of amplification in an  
20 automated DNA cycler; and
- 21 d)using products of said cycles of amplification and performing gel  
22 electrophoresis.

1 63. The method of claim 62, wherein the mixture is comprised of:

- 2 a) 50 mM KCl;
- 3 b) 10mM Tris-HCl with a pH of 8.3;
- 4 c) 1.5 mM MgCl<sub>2</sub>;
- 5 d) 0.5% NP40;
- 6 e) 0.5% Tween 20;
- 7 f) 200 mM each of deoxynucleoside triphosphates;
- 8 g) 2 mM of primer sets; and
- 9 h) 2 U of thermostable Taq DNA polymerase.

1 64. The method of claim 62, wherein the said number of cycles of amplification is 40.

1 65. The method of claim 62, wherein the said cycles of amplification are comprised of:

- 2 a) heating to 94°C for 1 minute to allow the DNA to denature;
- 3 b) cooling to 69°C for 1 minute to allow the primers to anneal; and
- 4 c) heating to 72°C for 2 minutes to allow for primer extension.